

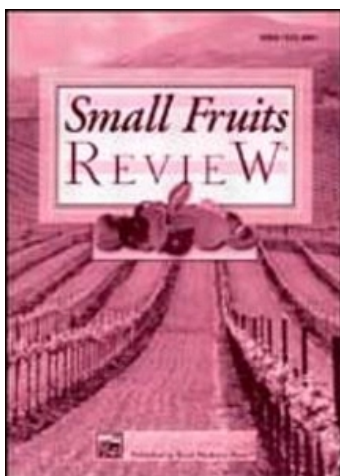
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Screenhouse Evaluations of a Mason Bee *Osmia ribifloris* (Hymenoptera: Megachilidae) as a Pollinator for Blueberries in the Southeastern United States

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SUMMARY. The behavioral traits and the pollination efficiency of *Osmia ribifloris* Cockerell (Hymenoptera: Megachilidae) were studied to assess the potential value of this bee for pollinating southern blue-

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berry crops (*Vaccinium* sect. *Cyanococcus*). Emergence for this typically univoltine bee can be made to coincide with blueberry flowering, but there were some unusual patterns. For example, some bees delayed prepupal development and emerged one year late (i.e., parsivoltinism). Adults began foraging at blueberry flowers at air temperatures as cool as 9°C. Thirty-three to 67 percent of open-pollinated rabbiteye blueberry (*Vaccinium ashei* Reade) flowers visited solely by female *O. ribifloris* set seeded fruits. Bagged flowers that excluded *O. ribifloris* resulted in 0-5% seedless fruit. Calculations based on flower visits and fruit set for female bees suggest that the monetary return a farmer receives from each female bee that nests in the orchard is about \$12.00 to \$24.00. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Web-site: <<http://www.HaworthPress.com>>]

KEYWORDS. Apoidea, non-*Apis* pollinator, *Vaccinium*, fruit set, pollination, parsivoltinism

INTRODUCTION

Mason bees (*Osmia* spp.) are among the most promising pollinators of North American fruit crops (e.g., blueberries, apple, pears, and sweet cherries, Bosch and Kemp, 2002; Drummond and Stubbs, 1997), but are rare at flowering rabbiteye blueberries (*Vaccinium ashei* Reade: Ericaceae) in the southeastern United States (Cane and Payne, 1988, 1990). *Osmia ribifloris* Cockerell is a mason bee that efficiently pollinates rabbiteye blueberries (Sampson and Cane, 2000; Sampson et al. 1995), but does not naturally co-occur with this cultivated blueberry. *O. ribifloris* populations can be easily relocated to rabbiteye blueberry orchards by first encouraging females to nest in man-made domiciles; cylindrical cavities drilled into wooden blocks or straws arranged inside cardboard boxes. After the brood reach maturity inside their cocoons, they can easily be recovered from the trap-nests and then transported to where they are needed (Stubbs et al., 1994; Torchio, 1990). Female *O. ribifloris* are not aggressive, so blueberry growers can comfortably handle bees without the need for protective bee suits. We report here on aspects of *O. ribifloris* biology that will help with developing the bee as a pollinator for rabbiteye and perhaps southern highbush blueberries. We have studied the regional and environmental adaptation, nest provisioning behavior, pollination efficiency, and monetary value for *O. ribifloris*.

MATERIALS AND METHODS

Study area, pollinator management, and regional adaptability. A population of *Osmia ribifloris* was maintained for four generations in a secure 15 m \times 9 m \times 5 m (L \times W \times H) screenhouse located at the USDA-ARS Small Fruit Research Station, Poplarville, MS. Cocoons stored for 90-120 days at 4-5°C were warmed at 21-29°C to break adult dormancy just before each release. Incubation conditions and seasonal emergence patterns for cocooned bees (i.e., emergence date, sex, parasites, and release date) were recorded daily for *O. ribifloris*. As part of the X-ray screening protocols for natural enemies, the parasitic wasps, *Monodontomerus* sp. and *Sapyga pumila* Cresson were removed, and healthy adult *Osmia ribifloris* transferred to the screenhouse for testing. Cocoons containing healthy prepupae (i.e., post defecating larvae) that had not emerged the first year were held a second year to test their viability. Methods for acquiring and managing wild bees were those described by Sampson and Cane (2000).

Environmental adaptation. Rates of flower visitation by female bees were recorded under favorable weather conditions between 8-15 March 2000. The number of *V. ashei* flowers visited by females was counted during 30-s foraging bouts. Each day, at least 15 bouts were tracked in the morning (0915-1030 HR) and in the afternoon (1304-1400 HR). One-way analysis of variance was used to test the hypothesis that female *O. ribifloris* foraged faster in the afternoons.

First counting male O. ribifloris cruising above the bushes gauged the daily onset of adult flight activity of bees released inside the screenhouse. Females were then counted as they visited *V. ashei* flowers. Bees were counted from 0700-1900 HR for 3 d, while corresponding weather readings were compiled by a portable weather station (GroWeather 7450/7455, Davis Instruments, Hayward, CA) in the screenhouse. Multivariate regression (MANOVA in PROC GLM, SAS Institute, 1985) identified which of four weather factors—air temperature (°C), percent relative humidity, solar radiation (W/m²), and barometric pressure (kPa)—correlated with bee activity.

Nest provisioning behavior. Nesting and foraging behaviors, which are difficult to measure in the field, were more easily studied inside the screenhouse. The average flowers visited per foraging trip were calculated from five uninterrupted foraging sequences between the time bees departed nests and returned. Sequences were monitored between 10-16 March 2000. The number of trips needed to provision a nest cell was tallied for six females over a 1 or 2-d period from 20-23 March 2000. Six

whole provision masses were frozen, later thawed and placed into pre-cleaned glass vials. Each vial contained 50 mL filtered ethanol and the resulting pollen suspension was dispersed in an ultrasonic bath. Half of the agitated subsample (25 mL) of the pollen suspension was counted using a particle counter (HIAC-ROYCO, Pacific Scientific Inc., Silver Spring, MD) (Cane et al., 1996). Doubling the pollen count gave an estimate of the total blueberry tetrads contained in a single *O. ribifloris* provision.

Of interest to those wanting to maintain healthy bee populations, propensities for nest reuse were evaluated for *O. ribifloris*. To test this, each nesting block (N = 15 blocks) contained six straws, two of which were clean and unused (controls), two were used before by *O. ribifloris*, and the remaining two were earlier used by *O. lignaria*. The contents of the used straws were pulverized and shaken in each straw. Large obstructions such as partitions and dead immature bees were removed from the used straws. The clean straws were sliced open and bound with clear, adhesive tape so as to duplicate as closely as possible the distortions caused by repairing the used straws before inserting them into the nesting blocks. The six straws were randomly assigned to the six holes in each block. If the used nest straws were overwhelmingly rejected by *O. ribifloris*, then the two additional nest blocks containing six clean straws were provided to alleviate possible nest usurpation (total nest straws = 102). A chi-square test tested if the selection of the three straw types (new, old *O. ribifloris*, and old *O. lignaria* straws) by female bees was random.

Pollination efficiency and monetary value. Bees were provided with five species of blueberry (N = 26 clones) native to the Gulf Coastal Plain (USDA hardiness zone 8B). They were *Vaccinium ashei* Reade, diploid and tetraploid *V. corymbosum* L., *V. darrowi* Camp, *V. elliotii* Chapm., and *V. tenellum* Aiton. Primary food sources of the caged bees were 300 potted rabbiteye blueberry plants. A minimum of 25 terminal racemes per blueberry species, most of which had between 6-26 flowers (N = 335 racemes), were chosen on these plants of all vigorous clones and their flowers counted. Pollination efficiency and the level of autogamy (= parthenocarpy) for each blueberry species were based on percent green fruit set, percent ripe fruit set, and percent fruit drop measurements using the same protocols developed by Sampson and Spiers (2002), except that green fruit set was evaluated 35 d after flowers were pollinated. The percent fruit drop was the percentage of green berries that were present on d 35, but did not mature. Ripe berries were harvested daily from tagged clusters from 5 May 2001 to 11 July 2001. Statistical

analyses of fruit set observations (see Table 1) were the same as those reported by Lyrene (1989), Sampson and Cane (2000), and Sampson and Spiers (2002).

RESULTS AND DISCUSSION

Regional adaptation. Adult *O. ribifloris* emerged after chilling from 22 February to 31 March during 1999–2002; this coincided with rabbiteye blueberry bloom in Mississippi. For some cocoons, unplanned incubation stimulated earlier adult emergence (28 January–2 February). Under some circumstances, early emergence might be beneficial to this bee's pollination of southern highbush blueberries–hybrid blueberries that can bloom a month before rabbiteye blueberries (Sampson and Spiers, 2002). Although *O. ribifloris* is univoltine, delays in emergence (i.e., parsivoltinism) also occurred. In an isolated case, 1 in 5 bees passed the first winter as prepupae, and the second winter as adults. Both sexes had similar rates of parsivoltinism ($P^2 = 0.29$; $df = 1$, $P = 0.5912$). Parsivoltinism in *Osmia* appears to be a rare, but facultative condition perhaps induced by unusual climactic or rearing conditions (Bosch and Kemp 2000; Torchio and Tepedino, 1982; Wcislo and Cane, 1996).

Although male *O. ribifloris* are the first bees to emerge in the spring in larger numbers, female bees are the more valuable blueberry pollinators. Female bees can control the sex of their offspring, and the pro-

TABLE 1. Summary of ANCOVA statistics. Effect of *Vaccinium* species (SP), level of pollinator (*O. ribifloris*) visitation (TRT: no visitation/virgin), and their interaction on the reproductive characteristics of blueberries pollinated by *Osmia ribifloris*.

Source	Green fruit set (%)			Ripe fruit set (%)			Fruit drop (%)		
	df	F	P	df	F	P	df	F	P
SP	5	38.37	**	5	19.58	**	4	1.69	NS
TRT	1	21.41	**	1	40.05	**	1	2.12	NS
SP*TRT	5	2.71	0	5	14.6	**	1	1.5	NS
Error df	312			311			113		

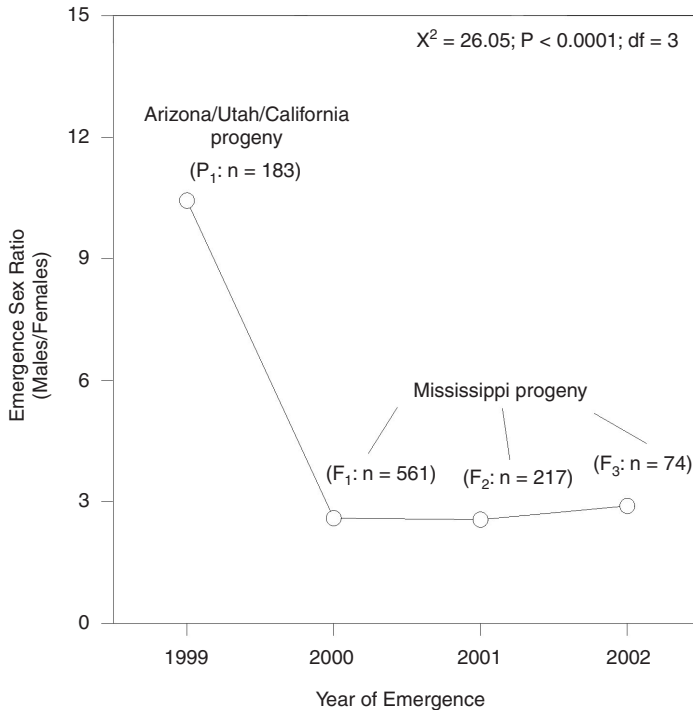
** $P \leq 0.001$, * $0.0001 < P \leq 0.05$, NS – $P > 0.05$.

duction of male-biased progeny distinguishes solitary bees like *Osmia* from many of the social bees that exist in larger female groups (Sugiura and Maeta, 1989; Torchio, 1985, 1990). Male-biased sex ratios for *O. ribifloris* were initially high for bees imported from the west, but quickly fell to a more stable sex ratio for succeeding generations reared primarily on *V. ashei* pollen (Figure 1) (Torchio, 1990). There are many intraspecific factors that affect sex ratios in *Osmia*: offspring size, seasonality, locality (Bosch and Kemp, 2002), dimensions of nest cavities (Torchio, 1990), nest cell size, and quantities of stored food (Krombien, 1967). Lowering or stabilizing male:female ratios is desirable for commercial blueberry pollination, as *O. ribifloris* females visit and pollinate substantially more blueberry flowers than males (Sampson, personal observation). Further studies are planned to identify the sources of these variable sex ratios, and develop methods to maximize female production.

Environmental adaptation. *O. ribifloris* flew during most of the daylight hours (0700-1800 HR), on clear, sunny days. Temperature and illumination influenced ($P < 0.003$) foraging activity. Females flew at temperatures as cool as 9°C (Figure 2). This is a threshold several degrees lower than that reported for a related fruit pollinator, *O. lignaria* (Torchio, 1985). Foraging peaked at 1300 HR as air temperatures also peaked near 21-26°C (Figures 2 and 3). Except for rain, the bees' foraging activities were not correlated with fluctuations in relative humidity and barometric pressure (Figures 2 and 3, $P > 0.5$).

O. ribifloris visited *V. ashei* blooms at a uniform rate throughout the day (4 ± 1 flowers every 30 s; $F = 0.73$; $df = 1, 136$, $P = 0.3944$). This rate equaled the rate of females foraging from northern highbush blueberries (*V. corymbosum*) in California (3-4 flowers/30s calculated from data published by Torchio, 1990). Females returned to their nests every 10.2 minutes ($SD = 2.7$, $N = 17$) with a scopal load of pollen gathered from an average of 55 *V. ashei* flowers (range: 43-63, $N = 5$). An egg being laid on the provision followed the completion of 18 pollen collecting trips ($SD = 2$, $N = 6$), and so, multiplying this number by the bees' average foraging tempo (55 flowers/trip), we can expect about 990 flowers were visited for each nest cell provisioned. Bees often needed 2 d to complete a nest cell. Partial nest provisions were often finished and, after receiving an egg, were sealed the next morning. Each provision contained a mixture of nectar and approximately $1.39 \times 10^6 \pm 1.60 \times 10^5$ blueberry tetrads ($N = 6$). Female *O. ribifloris* were not known to reuse natal nests for nest cell provisioning (Torchio, 1990) because the risk of fungal infections is greater. However, our findings indicate fe-

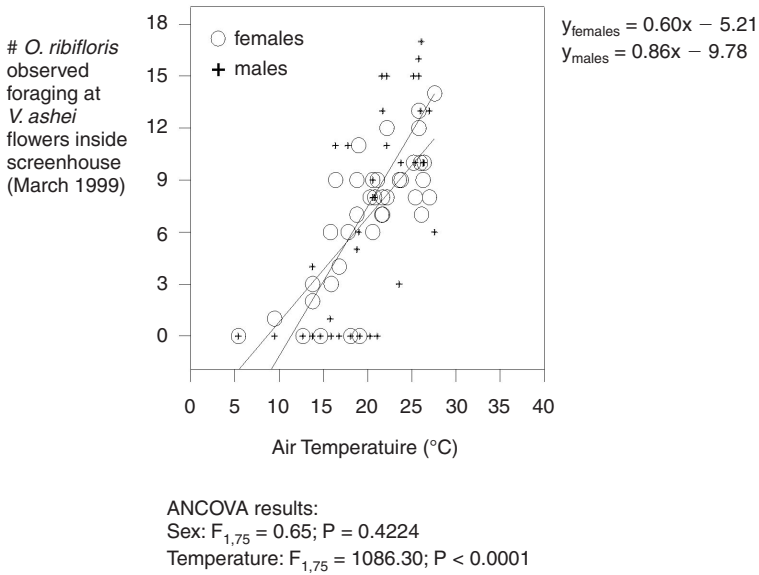
FIGURE 1. Emergence sex ratios (#males/#females) for *O. ribifloris* maintained in the screenhouse for four seasons (years 1999-2002). P_1 is the parental generation and $F_1 - F_3$ are the subsequent filial generations. The sample sizes (total adults emerged) for each generation and chi-square analysis are shown. Declining sample sizes were due to cocooned bees being repatriated for field trials in Oregon blueberry fields.



males cannot readily tell pristine nest straws from used straws ($X^2 = 1.143; df = 2; P > 0.05$). It is likely that older natal straws become more acceptable to females when their interiors were cleared of debris. Clean nesting materials should always be provided annually to *O. ribifloris* to maintain proper hygiene within nesting populations.

Pollination efficiency and pollination value. The potential value of *O. ribifloris* to blueberry production may be calculated from existing information. *O. ribifloris* pollinated cultivars of rabbiteye blueberries with an efficiency that equaled other established pollinator species in the Southeast (Cane and Payne, 1990; Payne et al., 1989, 1991; Sampson

FIGURE 2. The diel foraging activity of *O. ribifloris* as a function of ambient air temperature inside the screenhouse. Female (○) and male (+) responses to air temperature are separated. ANCOVA results and equations are shown for the two regressions.



and Cane, 2000). Mean overall green fruit set of rabbiteye blueberries in these trials was 43.3% (range: 33%-67%); ripe fruit set was 33.6% (range: 24%-56%, Table 1 and Figure 4). If 990 flowers are visited by a female per nest cell and 34% of them set ripe fruit, then for each nest cell produced, the foraging efforts of a female *O. ribifloris* will yield 336 blueberries. These blueberries would weigh approximately 0.60 kg, and be worth between \$1.00-\$2.00 at recent wholesale prices (Williamson and Lyrene, 1997). A female *O. ribifloris* can provision about 12 nest cells in her lifetime (Torchio, 1990). So, upon her death she would have contributed to the production of \$12.00-\$24.00 worth of fresh blueberries. This value is similar to that calculated for the southeastern blueberry bee, *H. laboriosa* (Cane, 1997). The above calculations, although simplistic, highlight the potential value of *O. ribifloris* as a pollinator of rabbiteye and perhaps southern highbush blueberries. Sustaining larger populations of *O. ribifloris* on the farm might also prove to be lucrative for southeastern blueberry producers. Currently, supplemental blue-

FIGURE 3. The diel flight activity of male and female *O. ribifloris*. Observed (○) and predicted (+) values are given. Model equations containing four terms [i.e., the four weather factors: temperature (temp), solar radiation (sun), relative humidity (RH), and barometric pressure (BP)] from which predicted values and regression lines were derived, are provided.

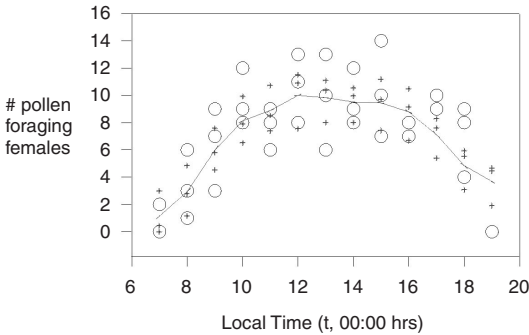
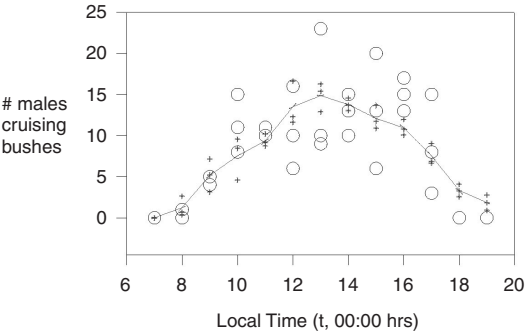
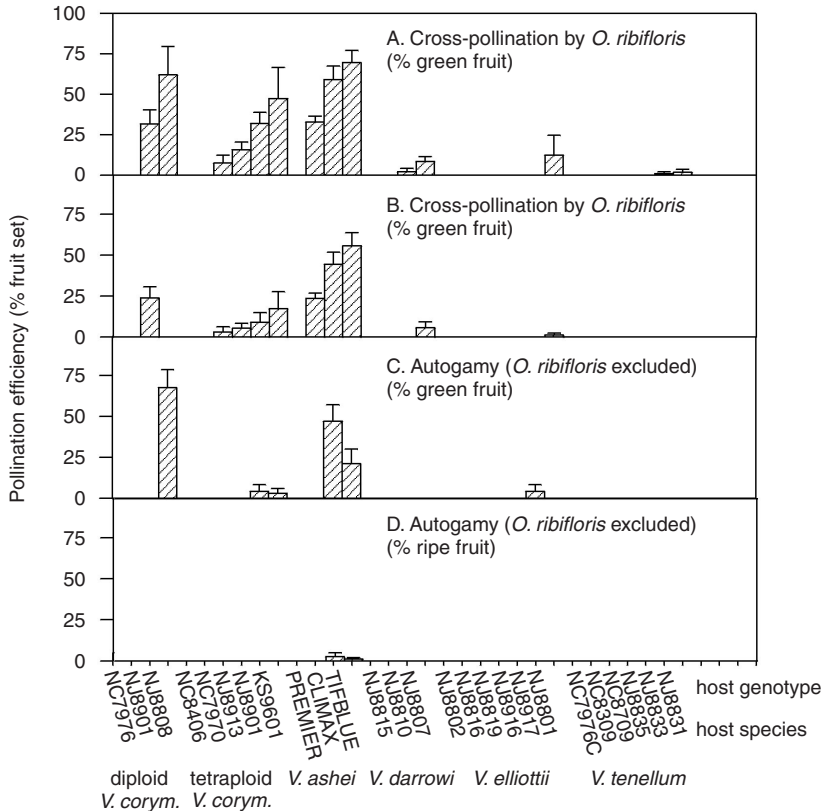


FIGURE 4. Percent of green and ripe fruit that set for tagged racemes resulting from two levels of visitation: open-pollination by *O. ribifloris* (4A, 4B), *O. ribifloris* excluded (4C, 4D). Fruit set values are shown for each species and the clones and cultivars comprising each species. Gaps indicate 0% fruit set for a given clone(s) and treatment(s). Bars represent ± 1 standard error.



berry pollinators (e.g., honey bees and bumble bees) are obtained from off-farm sources, often with greater inconvenience or expense to the grower. For a bee like *Osmia ribifloris*, the grower may own and annually propagate the bee on the farm (Batra, 1982; Sampson et al., 1995) and if diapausal mortality is low, and adult dispersal is minimized, the grower can recover and sell the surplus *O. ribifloris* cocoons.

Flowers of the two most popular *V. ashei* cultivars ‘Climax’ and ‘Tifblue’ set more fruit after being pollinated by *O. ribifloris* (Figure 4)

than those remaining unvisited. Unrestricted visitation yielded fruit sets that were no greater than the single-visit fruit set levels measured the year before (Sampson and Cane, 2000). Thus, a single visit by this bee is sufficient to set rabbiteye blueberry fruit. Northern and southern highbush blueberry clones (*V. corymbosum*) also received satisfactory pollination by *O. ribifloris*. Fruit sets might have been higher for *V. corymbosum* if an increased number of compatible plants were placed inside the screenhouse. Many clones of the wild blueberries, *V. elliotii*, *V. darrowi*, and *V. tenellum*, were largely ignored by *O. ribifloris*, were poorly pollinated (Figure 4), and, therefore, may not compete with cultivated blueberries for *O. ribifloris* pollinators.

CONCLUSIONS AND GROWER BENEFITS

We are moving closer to our goal of eventually releasing *O. ribifloris* as a blueberry pollinator in the Southeast. Field releases and pathological studies are now underway, and the final step is to expand our propagation efforts to produce enough bees for a limited field release. Experimental releases have occurred in Oregon with the objective of testing the nesting success of *O. ribifloris* in an open orchard environment. These data are forthcoming.

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